IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Richard B. Roth et al.

Serial No.: 10/661,966

Filing Date: September 11, 2003

Title: METHODS FOR IDENTIFYING SUBJECTS AT RISK OF MELANOMA AND TREATMENTS THEREOF

Examiner: Jehanne Souaya Sitton

Group Art Unit: 1634

Conf. No.: 9006

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Commissioner for Patents
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DECLARATION OF DR. CHARLES CANTOR UNDER 37 C.F.R. 1.132

Dear Sir:

I, Charles Cantor, declare as follows:

1. I have studied the human genome for a large part of my scientific career and a copy of my *curriculum vitae* is attached. I was Director of the Human Genome Center Project of the Department of Energy at Lawrence Berkeley Laboratory, and published the first textbook on genomics entitled *Genomics: The Science and Technology of the Human Genome Project*. I also was the Chair and Professor of the Department of Biomedical Engineering and Biophysics, and Director of the Center for Advanced Biotechnology, at Boston University. Before taking that position, I held professorship positions at Columbia University and the University of California, Berkeley. I have been the Chief Scientific Officer and Chairman of the Scientific Advisory Board of Sequenom, Inc. since 1998, and in May 2000 was appointed to the company's Board of Directors. I oversaw studies carried

out by the inventors that led to the scientific discoveries presented in the above-identified patent application.

2. I have reviewed the patent application and the rejection in the Office action dated September 18, 2006 based on a perceived lack of written description. I understand this perception may be addressed and overcome by showing the inventors had possession of the claimed methods at the time the patent application was filed. Below, I describe (i) methodology and discoveries presented in the patent application, (ii) genetic principles forming the basis for these discoveries, (iii) identification of another disease-associated locus using similar methodology, (iv) publicly available database information in support of the reported discoveries, and (v) other experimental evidence substantiating the discoveries. The data and methodology presented in the patent application and supporting evidence show the inventors had possession of the claimed methods at the time of filing.

3. Presented in the patent application is a genomic study in which many single nucleotide polymorphism (SNP) positions, spaced throughout the entire human genome, were typed in two populations: a melanoma population and a "healthy" control population. Certain variants occurring with a significantly high frequency in the cancer population were identified as markers residing in candidate loci associated with the disease. The inventors then verified that additional SNPs proximal to the incident marker polymorphism in each locus were significantly associated with the disease. Regions that contained multiple disease-associated polymorphisms were verified as being significantly associated with melanoma. One region identified by this method was a region encoding the BRAF protein, and the inventors typed additional polymorphic sites in this region. Of these, several polymorphisms were significantly associated with melanoma, and they were spaced across the studied region. This region, or "hot zone," is the region specified in amended claim 1. Thus, the inventors identified that the BRAF region was significantly associated with melanoma by (i) identifying a locus containing an incident polymorphic marker associated with the disease in a genome-wide scan, and (ii) verifying multiple polymorphic sites proximal to the incident marker in the locus also were associated with the disease.

4. Identifying a disease-associated region by this methodology is supported by accepted genetic principles. The concept of linkage disequilibrium in genetics embodies the phenomenon that a disease-associated region in the human genome contains a cluster of polymorphisms associated with a disease state. Specifically,

> markers very close to the disease gene will tend, more likely than average, to retain the haplotype of the original chromosome because, as the distance to the disease gene shrinks, it becomes less likely that recombination events will have occurred in this particular region.

From Cantor & Smith, *Genomics: The Science and Technology Behind the Human Genome Project*, 1999, John Wiley & Sons, Inc., New York, page 192. Thus, identifying multiple polymorphisms associated with a disease state within a region identifies the region as associated with the disease state. Accordingly, the inventors identified the claimed region as being associated with melanoma when they verified multiple polymorphic variants in the region were significantly associated with the disease.

5. Identifying a disease-associated region by this methodology is supported by the work of other researchers. Researches using similar methods independently located a complement factor H (CFH) gene region as being significantly associated with age-related macular degeneration (AMD). Klein *et al.*, like the inventors of the present patent application, screened SNPs spaced across the entire human genome in disease and control populations, and identified polymorphisms significantly associated with the disease[1]. Klein *et al.* then sequenced exons in the locus containing the associated SNPs and identified proximal polymorphisms associated with the disease. Edwards *et al.*, screened proximal polymorphisms in the CFH region and identified a sub-region particularly associated with the disease[2]. Haines *et al.* also identified the same CFH gene region as being associated with AMD from a 261 kilobase pair region containing a haplotype

associated with AMD[3]. Hageman *et al.* and Zareparsi *et al.* further confirmed the association of the CFH region with AMD[4,5].

6. I would like to point out that the inventors typed a significant number of polymorphisms in the BRAF region in the process of determining that the region was associated with melanoma. The following table shows, according to the BRAF region analyzed and claimed, (i) the number of polymorphisms the inventors analyzed, (ii) the number of polymorphisms in the HapMap database, (iii) the number of polymorphisms analyzed by the inventors overlapping with the HapMap database polymorphisms, (iv) the number of polymorphisms in the HapMap database having a minor allele frequency (MAF) of greater than 0.05, and (v) the number of polymorphisms analyzed by the inventors overlapping with the HapMap database polymorphisms having a MAF greater than 0.05.

Region	Inventor polymorphisms (IP)	HapMap polymorphisms (HP)	Overlap of IP and HP	HP with MAF >0.05 (HP>0.05)	Overlap of IP and HP>0.05
BRAF	12	73	10 (14%)	43	10 (23%)

In the claimed region, the inventors therefore analyzed 23% of the polymorphisms currently in the HapMap database having a MAF of greater than 0.05 at the time the present patent application was filed (i.e., September 11, 2003), which was more than three-and-a-half years ago. This degree of overlap with polymorphisms in the HapMap database is on par with, or better than, the overlap of polymorphisms Klein *et al.* analyzed for determining the association of CFH with AMD. Specifically, Klein *et al.* analyzed 13% of the CFH region SNPs in the HapMap database (analyzed 19 of 152 in the database; paragraph spanning pages 386-387). The inventors therefore identified the claimed BRAF region association with melanoma by analyzing a significant number of polymorphisms.

7. The inventors also collected further genetic data showing that the BRAF region was associated with melanoma. The inventors analyzed haplotypes within the claimed region and determined polymorphic variants associated with melanoma at positions 68398, 76779, 138875 and 146311 in SEQ ID NO: 1 were in strong linkage disequilibrium (LD).

These positions correspond to rs1267621, rs1267606, rs1267646 and rs1639679, respectively, and data is presented in paragraphs 00225 to 00228. These four polymorphic variants are located at the termini of the claimed region and the finding of strong LD provides evidence that the claimed region is significantly associated with melanoma. Also, the population sizes for the haplotype study are significant as there was a combined melanoma population of 1000 individuals and a combined control population of 898 individuals (e.g., Table 13).

8. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed in San Diego, California, on 25 July 2007.

Charles Cantor, Ph.D.

Documents cited:

1. Klein *et al.*, "Complement factor H polymorphism in age-related macular degeneration," *Science 308*: 385-389 (2005).

2. Edwards *et al.*, "Complement factor H polymorphism and age-related macular degeneration," *Science 308*: 421-424 (2005).

3. Haines *et al.*, "Complement factor H variant increases the risk of age-related macular degeneration," Science 308: 419-421 (2005).

4. Hageman *et al.*, "A common haplotypes in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration," *Proc. Natl. Acad. Sci. 102(20):* 7227-7232 (2005).

5. Zareparsi *et al.*, "Strong association of the Y402H variant in complement factor H at 1q32 with susceptibility to age-related macular degeneration," *Am. J. Hum. Genet. 77*: 149-153 (2005).

Charles R. Cantor, PhD

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Academic Qualifications: 1966-69, Asst. Prof. of Chemistry, Columbia Univ.; 1969-72, Assoc. Prof. of Chemistry, joint appointment in Biological Sciences, Columbia Univ.; 1972-81, Prof. of Chemistry, joint appointment in Biological Sciences, Columbia Univ.; 1981-89, Prof. and Chair of Genetics and Development, College of Physicians and Surgeons, Columbia Univ., Deputy Dir. for Education, 1981-85, Comprehensive Cancer Ctr., and Deputy Dir. for Biotechnology, 1985-88, Comprehensive Cancer Ctr.; 1988-89, Higgins Prof. of Genetics and Development, Faculty of Medicine, Columbia Univ.; 1988-90, Dir., Human Genome Ctr., Lawrence Berkeley Laboratory; 1989-91, Sr. Biochemist, Cell and Molecular Biology Div., Lawrence Berkeley Laboratory; 1989-92, Prof. of Molecular Biology, Univ. of CA, Berkeley; 1990-92, Principal Scientist, Human Genome Project, U.S. Dept. of Energy; 1991-92, Sr. Biochemist, Chemical Biodynamics Div., Lawrence Berkeley Laboratory; 1992-pres., Prof. of Biomedical Engineering, Boston Univ.; 1992-pres., Dir., Ctr. for Advanced Biotechnology, Boston Univ.; 1994-pres., Prof., Pharmacology Dept., Boston Univ. Medical School; 1995-98, Chair, Dept. of Biomedical Engineering, Boston Univ.; 1998-pres., Chief Scientific Officer, Sequenom, Inc.; 2002, Founder, SelectX Pharmaceuticals, Inc.; 2005, Founder, Retrotope, Inc.

Present Positions: Boston University, Boston, MA USA, co-director of the Center for Advanced Biotechnology and professor of Biomedical Engineering. Sequenom, Inc., San Diego, CA USA, Chief Scientific Officer, founder and member, Board of Directors.

Research Work and Funding: Large projects including Director of the Human Genome Center of the Department of Energy at Lawrence Berkeley Laboratory. 35 years of continuous research funding from government and private sources.

Awards and Honors:
1969-71, Fellow of the Alfred P. Sloan Foundation; 1972, Fresenius Award in Chemistry; 1973-74, Guggenheim Fellow; 1975-76, Fairchild Distinguished Visiting Scholar, CA Inst. of Technology; 1978, Eli Lilly Award in Biological Chemistry; 1981, Fellow of the Amer. Assoc. for the Advancement of Science; 1985, Outstanding Investigator Grant, Natl. Cancer Inst.; 1988, Biochemical Analysis Prize of the German Society of Clinical Chemistry; 1988, Member of the Natl. Acad. of Sciences; 1988, Member of the Amer. Acad. of Arts and Sciences; 1989, ISCO Award for Advances in Biochemical Instrumentation; 1990, Herbert A. Sober Award, Amer. Society for Biochemistry and Molecular Biology; 1990, Honorary Member, Japanese Biochemical Society; 1992, Fellow of the CA Acad. of Sciences; 1993, Fellow of the Biophysical Society; 2000, Emily M. Gray Award from the Biophysical Society; 2002, Chief Scientist of the Year, T Sector and BIOCOM; 2004, Ohio State University Human Cancer Genetics Program Commemorative Medal for Excellence in Research and Clinical Care

Selected Publications since 2004:

Smylie, K.J, Cantor, C.R., and Denissenko, M. 2004. *Analysis of Sequence Variations in Several Human Genes using Phosphoramidite Bond DNA Fragmentation and Chip-based MALDI-TOF.* Genome Research, 14, 134-141.

Karaoz, U., Murali, T.M., Letovsky, S., Zheng, Y., Ding, C., Cantor, C.R., and Kasif, S. 2004. *Whole Genome Annotation Using Evidence Integration in Functional Linkage Networks.* Proc. Nat. Acad. Sci. USA, 101, 9, 2888-2893.

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Selected Recent Professional Affiliations and Service:

1976-pres., Series Editor, Advanced Textbooks in Chemistry, Springer-Verlag, New York; 1978-pres., Biophysical Society (Council Member, 1978-81); 1982-pres., Amer. Society of Biochemistry and Molecular Biology; 1984-pres., Nomenclature Commission of the Intl. Union of Biochemistry and Molecular Biology; 1985-pres., Scientific Advisory Council, Roswell Park Memorial Inst.; 1988-pres., Biomedical Advisory Committee, Pittsburgh Supercomputing Ctr.; 1988-pres., Cell and Membrane Transport Commission, Intl. Union of Pure and Applied Biophysics; 1988-pres., Member, Exec. Committee and Founding Council, Intl. Human Genome Organization (HUGO) and 1990-2006, Vice President; 1991-95, Chair, HUGO Human Genome Mapping Committee (HGMC); 1992-1996, President, HUGO Americas; 1988-pres., Editorial Board, *Current Opinion in Biotechnology;* 1992-97, Member, Board of Dir., Chair, Scientific Advisory Board, ATGC/AT Biochem, Inc.; 1992-02, Member, Advisory Board, Boston Univ. Journal of Science Technology and Law; 1994-pres., Co-chair, Biotechnology Advisory Committee, Fisher Scientific; 1996-pres., Member, Editorial Board, Biotechniques; 1996-97, Member, NRC Committee, "Bits of Power"; 1997-98, Member, DARPA Advisory Committee on Biological Warfare Defense; 1996-2000, Member, Advisory Board, Encyclopedia of Molecular Biology; 1996-00, Member, FASEB Consensus

Committee on Federal Funding, representing the Biophysical Society; 1997-2000, Quest Scholar, Quest Diagnostics, Inc., San Juan Capistrano, CA; 1998-2000, Member, Defense Intelligence Agency Bio 2020 Red Team, Washington, D.C.; 2000-pres., Adjunct Prof., Dept. of Bioengineering, UCSD; 2001-pres., Science Advisory Board, Brandeis Univ. School of Science; 2002, Advisory Committee Member, Stockholm Strategic Research Foundation; 2002-pres., Scientific Advisory Board, Buffalo Ctr. of Excellence in Bioinformatics; 2003-pres., Member, National Advisory Board, Boston University Research Center for Translational Genomics and Human Rights, Boston, MA; 2004-pres., Scientific Advisory Board, Joint Center for Structural Genomics, La Jolla, CA; 2004-pres., Scientific Advisory Board, Uppsala Bio-X, Uppsala, Sweden Currently Board member or advisor for 20 or more companies including Fisher Scientific, US, Techno Ventures Management (Germany), Strand Genomics (India). 44 issued U.S. patents.